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STREPTOCOCCI OCCURRING AS DIPLOCOCCI IN RATS.
(*M. norvegicus*.)*

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THE most striking features of the following infection encountered in two rats are the presence in the affected tissues of numerous diplococci morphologically resembling the meningococcus; diplococcemia, and the presence of a marked subcutaneous edema.

While the micrococci occur mainly in pairs, occasionally short chains may be detected in smears from the tissues. They take the ordinary aniline dyes readily, and retain the stain in Gram's method. They grow readily upon transfer from the tissues to +1 agar slants.

Their biochemical characters, as far as determined, may be summed up briefly as follows: Upon +1 agar slants, in 24 hours, at 37°, pin-point to 0.25 mm. circular, white colonies with thin even edges appear. In 48 hours the colonies are larger and finely granular under low magnification, with finely notched or smooth edges. Colonies upon +1 rabbit's blood-agar plates are surrounded by a zone of hemolysis; the growth is more delicate upon rabbit's blood-agar slants than upon plain agar. Cultures may be kept viable for months on rabbit's blood agar, but not so on plain agar. Slight flocculent growth in +1 broth. Delicate white growth, without liquefaction, in +1.5 gelatin (18-25°) in the case of culture 2,854; no growth in the case of culture 22,398. No visible change in +1 milk during seven days' growth. Acid is produced in +1 litmus broth, containing 1 per cent dextrose, levulose, saccharose, maltose, and galactose; mannite and inulin are not fermented. Culture 2,854 produces acid from lactose while 22,398 does not. As usual the growth is very much more luxuriant and longer chains are formed in the media containing fermentable carbohydrates. Experimentally the micro-organisms were pathogenic to white rats but not to guinea-pigs nor rabbits.

Notes on the original infected rats read as follows:

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I. ADULT FEMALE *M. norvegicus* 22,398.

All the subcutaneous tissues are infiltrated with a marked sero-gelatinous edema, otherwise no noticeable abnormality is shown. Brain and serous membranes are normal. Smears from the heart's blood show enormous numbers of meningococcus-like diplococci, not seen in phagocytes; also trypanosomata which are alive in fresh preparations. Numerous isolated colonies of the cocci were obtained on +1 agar slants.

Animal inoculations.—A guinea-pig inoculated intraperitoneally with a physiological salt solution emulsion of the heart's blood remained well for a month. No trypanosomes appeared in the heart's blood. Chloroformed and found normal.

An adult white rat inoculated intraperitoneally with +1 broth emulsion of a 48-hour +1 agar slant culture, grown directly from the heart's blood of rat 22,398 was chloroformed six days later and found perfectly normal.

An adult white rat inoculated intraperitoneally with a +1 broth emulsion of a +1 rabbit's blood-agar slant culture, two removes from the heart's blood of rat 22,398, died in less than 24 hours. It showed subcutaneous injection, and a small amount of bloody fluid in peritoneal cavity; spleen, liver, kidneys, testicles, and lungs were intensely congested; fibrinous specks on pleura of lungs; serous exudation in thorax, brain congested, spinal cord normal. Microscopically diplococci were very numerous in the peritoneal exudate and occurred chiefly within phagocytes; here a few chains of two to four diplococci were seen. Numerous cocci in diplococcus form only were seen in preparations from the spleen and pleural exudate. None were seen in smears of the heart's blood. The micro-organism was recovered from the peritoneal exudate, spleen, and heart's blood.

An adult white rat inoculated subcutaneously with a +1 rabbit's blood-agar slant culture, two removes (48 hours) from the heart's blood of rat 22,398, appeared sick for a day after the injection but recovered and was chloroformed and found normal four days later.

A half-grown Belgian hare was inoculated intravenously with a broth emulsion of one rabbit's blood-agar slant culture, two removes (48 hours) on blood agar from heart's blood of rat 22,398. It

remained well and was chloroformed and found normal four days later.

The animals were chloroformed and examined in so comparatively short a time after inoculation owing to my transfer from San Francisco to Oakland.

II. ADULT PREGNANT *M. norvegicus* 2,854.

This rat was caught and kept alive in a cage for four days when it died. It had a wound on the anterior aspect of left thigh. On dissection the subcutaneous tissues of the whole left half of the body were found infiltrated with a sero-gelatinous exudate and appeared somewhat hyperemic. The left inguinal glands were enlarged to 4-5 mm. in diameter, were firm and white on section, and imbedded in a sero-gelatinous periglandular exudate. The submaxillary and right and left cervical glands presented a similar appearance. The exudate also extended down the left thigh. The right side of the body was not so affected. The peritoneal cavity contained some fluid.

Smears from the affected glands showed enormous numbers of biscuit-shaped diplococci, rarely in leucocytes, and rarely in chains of four to six diplococci. No micro-organisms were found in preparations from the spleen and heart's blood.

Pure cultures of the cocci were obtained from the left inguinal gland on rabbit's blood-agar plates. Many colonies of similar cocci also grew on agar slants inoculated from the peritoneal fluid, spleen, and heart's blood.

Animal inoculations.—A broth emulsion of the left inguinal gland was used to inoculate two guinea-pigs, one subcutaneously, the other intraperitoneally; a white rat subcutaneously; a wild rat (*M. norvegicus*) subcutaneously; a Belgian hare intravenously. The guinea-pigs and hare were chloroformed 23 days later in a healthy condition and found normal. The wild rat died in 23 days from some unknown cause.

The white rat died in 15 days. There was an abscess at the point of inoculation; marked general subcutaneous congestion with sero-gelatinous exudation in both groins; abdomen distended with much bloody fibrino-purulent exudate and abdominal organs bound together

with the same. Cocci were very numerous in the peritoneal exudate and here the formation of chains was more marked than in the original rat. Probably the inoculation was partly intraperitoneally.

F. Kutschera¹ described cultures isolated from white mice which succumbed to a streptococcus epizootic. Owing to the differences in cultural procedures adopted by this worker and myself, one can only point to the following, differential characters between the two strains: In the epizootic described by Kutchera, the lesions were essentially of a suppurative character, due to a mixed staphylococcus and streptococcus infection; the streptococci were pathogenic for white mice, white rats, and rabbits, while guinea-pigs were refractive. The streptococci isolated from this form of infection in *M. norvegicus* occurred almost entirely as kidney-shaped diplococci in the tissues of the naturally infected animals; the infection was characterized by a marked subcutaneous edema and sero-gelatinous exudate; the cocci were non-pathogenic for rabbits as well as for guinea-pigs.

¹ *Centralbl. f. Bakt.*, Orig., 1908, 46, p. 671.